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FILE 'REGISTRY' ENTERED AT 09:57:29 ON 04 APR 2006

=> S RESTRICTION ENZYME/CN
L1             1 RESTRICTION ENZYME/CN

FILE 'CAPLUS' ENTERED AT 09:57:42 ON 04 APR 2006

=> S L1;S RESTRICTION(W)(ENZYME OR ENDONUCLEASE);S METHYLASE
L2          4015 L1


          101305 RESTRICTION
           13259 RESTRICTIONS
          113732 RESTRICTION
                   (RESTRICTION OR RESTRICTIONS)
          767758 ENZYME
          444299 ENZYMES
          971104 ENZYME
                   (ENZYME OR ENZYMES)
           27746 ENDONUCLEASE
            8293 ENDONUCLEASES
           32206 ENDONUCLEASE
                   (ENDONUCLEASE OR ENDONUCLEASES)
L3         32920 RESTRICTION(W)(ENZYME OR ENDONUCLEASE)


            2844 METHYLASE
             722 METHYLASES
L4          3069 METHYLASE
                   (METHYLASE OR METHYLASES)

=> S L2,L3
L5         33241 (L2 OR L3)

=> S DOMAIN;S CLEAVAGE;S SPECIFICITY
          264347 DOMAIN
          137637 DOMAINS
L6        332367 DOMAIN
                   (DOMAIN OR DOMAINS)


          205853 CLEAVAGE
            5476 CLEAVAGES
L7        208436 CLEAVAGE
                   (CLEAVAGE OR CLEAVAGES)


          182878 SPECIFICITY
           18500 SPECIFICITIES
L8        194535 SPECIFICITY
                   (SPECIFICITY OR SPECIFICITIES)

=> S L7(W)L8;S L8(W)L6
L9           743 L7(W)L8


L10           87 L8(W)L6

=> S L9 AND L10
L11            1 L9 AND L10

=> D CBIB ABS
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L11 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2006 ACS on STN
2004:936088 Document No. 141:389850 Methods for altering the **cleavage specificity** of a type IIG restriction endonuclease. Xu, Shuang-Yong; Kobbe, Daniela; Zhu, Zhenyu; Samuelson, James (USA). U.S. Pat. Appl. Publ. US 2004219584 A1 20041104, 34 pp., Cont.-in-part of U.S. Ser. No. 150,028, abandoned. (English). CODEN: USXXCO. APPLICATION: US 2004-800946 20040315. PRIORITY: US 2000-2000/693146 20001020; US 2002-2002/150028 20020517.

AB The current invention provides methods for altering the **cleavage specificity** of a type IIG restriction endonuclease. Type IIG restriction endonuclease contains a cleavage domain adjacent to a methylase domain, the methylase domain located adjacent to a **specificity domain**. The method includes ligating a first DNA sequence and a second DNA sequence to form a fusion DNA , wherein (i) the first DNA sequence comprises a DNA segment encoding a catalytic domain and an N-terminal portion of a methylase domain of a first Type IIG restriction endonuclease, and (ii) the second DNA sequence, comprises a DNA segment encoding a **specificity domain** and a C-terminal portion of a methylase domain of a second Type IIG restriction endonuclease; such that the ligation occurs between sequences encoding the methylase domain. The host cell is transformed with fusion DNA to express a Type IIG restriction endonuclease with altered **cleavage specificity**.

=> S IIG OR (II(W)G)
174 IIG
8 IIGS
182 IIG
(IIG OR IIGS)
2075575 II
904 IIS
2076091 II
(II OR IIS)
2843042 G
1042 II(W)G
L12 1222 IIG OR (II(W)G)

=> S L12 AND L5
L13 8 L12 AND L5

=> S L13 NOT L11
L14 7 L13 NOT L11

=> D 1-7 CBIB ABS

L14 ANSWER 1 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN
2006:31394 Document No. 144:122710 Method for identifying, analyzing and cloning nucleic acid fragments for identification of genes and genetic elements and gene expression profiling. Harbers, Matthias; Shibata, Yuko (Kabushiki Kaisha Dnaform, Japan). PCT Int. Appl. WO 2006003721 A1 20060112, 70 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2004-JP9862 20040702.

AB The present invention provides a method for identifying, analyzing and cloning nucleic acid fragments for gene identification and expression profiling. It also provides a means for preparation of a new type of sequence tag, the GSC-tag (Gene Scanning CAP Anal. Gene Expression) tag which allows identification and characterization of nucleic acid mols. by their end sequences. Furthermore, GSC-

tags are prepared in such a way that related tags from the same nucleic acid mol. are combined in the same GSC-tag and that the spacer sequences connecting the two tags from the ends would allow for the labeling of the GSC-tag by a short sequence tag. Futhermore, the invention involves cloning of the tags derived from the DNA mols. Such tags are purified and cloned as concatamers into tag libraries for easier manipulation and sequencing (GSC library). Thus, the invention provides a means for high throughput sequencing of tags derived from the ends of nucleic acid mols. The method comprises preparing DNA fragments comprising sequences corresponding to two opposite end regions of a linear DNA mol. A linear DNA mol. is prepared from a nucleic acid and linkers are ligated to opposite ends of the linear DNA mol., wherein such linkers contain a cloning site and a recognition site for a **restriction endonuclease** that cleaves at a site outside its recognition site and within the linear DNA mol. The linear DNA mol. is circularized followed by digestion with a **restriction endonuclease** that cleaves at a site outside its recognition site. Hence, the DNA fragment is isolated which comprises opposite end regions of the linear DNA mol. Means of high value to studies including, but not limited to, expression profiling, splicing, promoter identification, identification of genetic elements, and beyond, which are essential components of com. applications and services including, but not limited to, drug development, diagnostics, or forensic studies are also provided.

L14 ANSWER 2 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN

2005:553088 Document No. 144:249362 Saturation mutagenesis of Thr862, the amino acid essential for substrate specificity of Eco57I **restriction endonuclease**. Rimseliene, R.; Janulaitis, A. (Institute of Biotechnology, Vilnius, LT-02241, Lithuania). Biologija (1), 11-14 (English) 2005. CODEN: BOLOE8. ISSN: 1392-0146. Publisher: Lietuvos Mokslu Akademijos Leidykla.

AB Type IIG **restriction endonuclease** (RE) Eco57I cleaves DNA 16/14 nucleotides away from the asym. recognition sequence 5'-CTGAAG. The enzyme also possesses methyltransferase activity that modifies the second A base within the 5'-CTGAAG strand of the target duplex (underlined). In previous studies, Eco57I mutants with altered substrate specificity 5'-CTGRAG were isolated. These mutant enzymes have Asn or Ser instead of Thr in the 862th position of the protein. In order to evaluate the impact of T862 on the substrate specificity, it was changed to the other 17 amino acids. The in vivo cleavage activity and substrate specificity of the resulting mutant enzymes was examined (i) by testing lethality of the mutants to the host cells in the absence or presence of Eco57I (specificity 5'-CTGAAG) and GsuI (specificity 5'-CTGGAG) methyltransferases, and (ii) by testing the ability of the mutants to induce SOS DNA repair response in the absence or presence of protecting methyltransferases. The results indicate that mutants T862G, T862C and, probably, T862A and T862D could display altered substrate specificity. The recognition sequence of T862F, H, K, L, Q, M and Y mutants was the same as that of the wild type enzyme. The remaining substitutions rendered the enzyme catalytically inactive.

L14 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN

2004:516400 Document No. 142:88561 S-Adenosyl-L-methionine-dependent **restriction enzymes**. Sistla, Srivani; Rao, Desirazu N. (Department of Biochemistry, Indian Institute of Science, Bangalore, India). Critical Reviews in Biochemistry and Molecular Biology, 39(1), 1-19 (English) 2004. CODEN: CRBBEJ. ISSN: 1040-9238. Publisher: Taylor & Francis, Inc..

AB A review. Restriction-modification (R-M) enzymes are classified as type I, II, III, and IV, based on their recognition sequence, subunit composition, cleavage position, and cofactor requirements. While the role of S-adenosyl-L-methionine (AdoMet) as the Me group donor in the methylation reaction is undisputed, its requirement in DNA cleavage reaction has been subject to intense study. AdoMet is a prerequisite for the DNA cleavage by most type I enzymes known so far, with the exception of R.EcoR124I. A number of new type II **restriction enzymes**

belonging to the type IIB and IIG family were found to show AdoMet dependence for their cleavage reaction. The type III enzymes have been found to require AdoMet for their restriction function. AdoMet functions as an allosteric effector of the DNA cleavage reaction and has been shown to bring about conformational changes in the protein upon binding.

L14 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN

2004:450197 Document No. 141:152946 Significance of codon usage and irregularities of rare codon distribution in genes for expression of BspLU11III methyltransferases. Kirienko, N. V.; Lepikhov, K. A.; Zheleznaya, L. A.; Matvienko, N. I. (Institute of Protein Research, Russian Academy of Sciences, Moscow Region, Pushchino, 142290, Russia). Biochemistry (Moscow, Russian Federation)(Translation of Biokhimiya (Moscow, Russian Federation)), 69(5), 527-535 (English) 2004. CODEN: BIORAK. ISSN: 0006-2979. Publisher: MAIK Nauka/Interperiodica Publishing.

AB Genes of adenine-specific DNA-methyltransferase M.BspLU11IIIa and cytosine-specific DNA-methyltransferase M.BspLU11IIIb of the type IIG BspLU11III restriction-modification system from the thermophilic strain Bacillus sp. LU11 were expressed in E. coli. They contain a large number of codons that are rare in E. coli and are characterized by equal values of codon adaptation index (CAI) and expression level measure (E(g)). Rare codons are either diffused (M.BspLU11IIIa) or located in clusters (M.BspLU11IIIb). The expression level of the cytosine-specific DNA-methyltransferase was increased by a factor of 7.3 and that of adenine-specific DNA only by a factor of 1.25 after introduction of the plasmid pRARE supplying tRNA genes for six rare codons in E. coli. It can be assumed that the plasmid supplying minor tRNAs can strongly increase the expression level of only genes with cluster distribution of rare codons. Using heparin-Sepharose and phosphocellulose chromatog. and gel filtration on Sephadex G-75 both DNA-methyltransferases were isolated as electrophoretically homogeneous proteins (according to the results of SDS-PAGE).

L14 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN

2003:1007142 Document No. 140:54479 Method for obtaining the 5'end of mRNA for cloning and analysis. Hayashizaki, Yoshihide; Carninci, Piero; Harbers, Matthias T. (Riken Corp., Japan; Kabushiki Kaisha Dnaform). PCT Int. Appl. WO 2003106672 A2 20031224, 121 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-JP7514 20030612. PRIORITY: JP 2002-171851 20020612; JP 2002-235294 20020812.

AB A method is disclosed for obtaining the 5'ends of transcribed regions from a plurality of nucleic acid fragments obtained from biol. materials or synthetic pools. The method comprises attaching a linker to nucleic acids and cleaving the nucleic acid with a **restriction enzyme** having its recognition site within the linker and its cleavage site within the nucleic acid corresponding to the 5' end of the mRNA. MRNA or cDNA/RNA hybrids can be selected using the 5' cap structure of the mRNA. In addition, methods of the invention may include preparing nucleic acids using a selective binding substance that is attached to a support. DNA fragments encoding the 5'ends are enriched for their individual anal. or for the anal. of concatemers thereof. The sequence information derived from 5' ends can be used for characterization and cloning of the transcriptome. These methods are also claimed for the development of diagnostic tools, including development of a reagent or a kit. An example describes the cap trapper method for full-length cDNA selection. A double-stranded linker having a recognition site for **restriction enzymes** MmeI, XhoI, I-CeuI, and XmaJI was designed and ligated to

single-stranded cDNA for preparation of 5' end specific tags. Another example tells of preparation of 5' end specific tags that are di-tags. DNA concatemers can be formed and characterized by the structure of the dimeric tags and the flanking linker sites. The 5' end sequence tags can be identified by a manual sequence anal. or by an automated process.

L14 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN
1999:39742 Document No. 130:249607 Phylogenetic relationships of the mitochondrial genomes in the genus Glycine subgenus Soja. Kanazawa, Akira; Tozuka, Azumi; Akimoto, Shin-ichi; Abe, Jun; Shimamoto, Yoshiya (Laboratory of Plant Genetics and Evolution, Hokkaido University, Sapporo, 060-8589, Japan). Genes & Genetic Systems, 73(4), 255-261 (English) 1998. CODEN: GGSYF5. ISSN: 1341-7568. Publisher: Genetics Society of Japan.
AB Restriction fragment length polymorphisms (RFLPs) of mitochondrial DNA (mtDNA) of wild and cultivated soybeans were analyzed to study their phylogenetic relationships. The observed number of differences in hybridization profiles greatly varied (1-17 patterns) with both the mtDNA probes and the **restriction enzymes** that were used. A cladistic anal. was conducted based on the RFLP data. In the parsimonious tree, four distinct groups appeared among 20 accessions of the subgenus Soja representing 20 mitochondrial genome types. Common features with regard to geog. distributions in natural populations in East Asia were observed among the mitochondrial genome types of wild soybean that belonged to the same group: one clade consisted of genome types **IIg** and VIIg that are detected with very low frequencies; another clade consisted of genome types Ic, Id, Ie, and Ik whose distributions are highly biased mainly in Japan. The genome types that are widely distributed in East Asia such as IVa, IVb, and Va were not grouped into the same clade. The mitochondrial genome types IIIb, IVb, and IVc, in which two different chloroplast genome types exist, belonged to the same clade. Possible changes in mitochondrial genomes during the expansion of the distribution of wild soybeans in East Asia were discussed.

L14 ANSWER 7 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN
1992:445794 Document No. 117:45794 The Xmn I site (-158, C→T) 5' to the Gγ gene: correlation with the Senegalese haplotype and Gγ globin expression. Ballas, S. K.; Talacki, C. A.; Adachi, K.; Schwartz, E.; Surrey, S.; Rappaport, E. (Dep. Med., Thomas Jefferson Univ., Philadelphia, PA, 19107, USA). Hemoglobin, 15(5), 393-405 (English) 1991. CODEN: HEMOD8. ISSN: 0363-0269.
AB There are 3 major African haplotypes associated with the sickle mutation: Benin (#19), Senegalese (#3), and Central African Republic (#20). Previous studies have suggested that the XmnI site (-158 bp 5' to the Gγ gene) is associated with elevated levels of Gγ and with the Senegalese haplotype, while other investigators questioned this association In order to clarify the issue, β haplotypes were determined, the presence of the XmnI site was tested for, and Hb F and Gγ expression levels were measured in 143 American Black patients with sickle cell anemia. Haplotypes were determined using 8 polymorphic sites in the β-like globin gene cluster: HincII 5' to ε, HindIII in IVS-**II** G.gamma. and Aγ, HincII within and 3' to ψβ, AvaII in IVS-II of β, and HpaI and BamHI 3' to β. The Gγ/Aγ ratio was analyzed by high performance liquid chromatog. using a C18 column. The XmnI site was present in all 31 chromosomes with the Senegalese haplotype. Of the remaining 255 chromosomes with other haplotypes, only 2 (0.8%) had the XmnI site present. There was significant correlation between the presence of the XmnI site and increased Gγ/Aγ ratio in a dose-dependent manner. The Hb F level was not significantly increased in the presence of the XmnI site. The data indicate that the XmnI site maintains a Gγ/Aγ ratio typical of fetal life but does not necessarily cause elevation of Hb F. The latter seems to depend on factors other than the XmnI site.

=> S IIF OR (II(W)F)

```
           823 IIF
             4 IIFS
           827 IIF
                 (IIF OR IIFS)
       2075575 II
           904 IIS
       2076091 II
                 (II OR IIS)
        593236 F
           417 II(W)F
L15       1241 IIF OR (II(W)F)

=> S L15 AND L5
L16         10 L15 AND L5

=> S L16 NOT L14
L17         10 L16 NOT L14

=> D 1-10 CBIB ABS

L17  ANSWER 1 OF 10  CAPLUS  COPYRIGHT 2006 ACS on STN
2006:183320  Biochemical and mutational analysis of EcoRII functional domains
     reveals evolutionary links between restriction enzymes
     . Tamulaitis, Gintautas; Mucke, Merlind; Siksnys, Virginijus (Institute
     of Biotechnology, Vilnius, LT-02241, Lithuania). FEBS Letters, 580(6),
     1665-1671 (English) 2006. CODEN: FEBLAL. ISSN: 0014-5793. Publisher:
     Elsevier B.V..
AB   The archetypal Type IIE restriction endonuclease EcoRII is a dimer that has a
     modular structure. DNA binding studies indicate that the isolated C-terminal
     domain dimer has an interface that binds a single cognate DNA mol. whereas the N-
     terminal domain is a monomer that also binds a single copy of cognate DNA.
     Hence, the full-length EcoRII contains three putative DNA binding interfaces: one
     at the C-terminal domain dimer and two at each of the N-terminal domains.
     Mutational anal. indicates that the C-terminal domain shares conserved active
     site architecture and DNA binding elements with the tetrameric restriction enzyme
     NgoMIV. Data provided here suggest possible evolutionary relationships between
     different subfamilies of restriction enzymes.


L17  ANSWER 2 OF 10  CAPLUS  COPYRIGHT 2006 ACS on STN
2005:326257  Document No. 143:3212  Conversion of the Tetrameric
     Restriction Endonuclease Bse634I into a Dimer:
     Oligomeric Structure-Stability-Function Correlations. Zaremba, M.;
     Sasnauskas, G.; Urbanke, C.; Siksnys, V. (Institute of Biotechnology,
     Vilnius, LT-02241, Lithuania). Journal of Molecular Biology, 348(2),
     459-478 (English) 2005. CODEN: JMOBAK. ISSN: 0022-2836. Publisher:
     Elsevier B.V..
AB   The Bse634I restriction endonuclease is a tetramer and belongs to the type IIF
     subtype of restriction enzymes. It requires two recognition sites for its
     optimal activity and cleaves plasmid DNA with two sites much faster than a
     single-site DNA. We show that disruption of the tetramerization interface of
     Bse634I by site-directed mutagenesis converts the tetrameric enzyme into a dimer.
     Dimeric W228A mutant cleaves plasmid DNA containing one or two sites with the
     same efficiency as the tetramer cleaves the two-site plasmid. Hence, the
     catalytic activity of the Bse634I tetramer on a single-site DNA is down-regulated
     due to the cross-talking interactions between the individual dimers. The
     autoinhibition within the Bse634I tetramer is relieved by bridging two DNA copies
     into the synaptic complex that promotes fast and concerted cleavage at both
     sites. Cleavage anal. of the oligonucleotide attached to the solid support
     revealed that Bse634I is able to form catalytically competent synaptic complexes
     by bridging two mols. of the cognate DNA, cognate DNA-miscognate DNA and cognate
     DNA-product DNA. Taken together, our data demonstrate that a single W228A
     mutation converts a tetrameric type IIF restriction enzyme Bse634I into the
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orthodox dimeric type IIP **restriction endonuclease**. However, the stability of the dimer towards chemical denaturants, thermal inactivation and proteolytic degradation are compromised.

L17 ANSWER 3 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
2005:145011 Document No. 143:281578 A homology model of **restriction endonuclease** SfiI in complex with DNA. Chmiel, Agnieszka A.; Bujnicki, Janusz M.; Skowronek, Krzysztof J. (Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology, Warsaw, 02-109, Pol.). BMC Structural Biology, 5, No pp. given (English) 2005. CODEN: BSBMBB. ISSN: 1472-6807. URL: http://www.biomedcentral.com/content/pdf/1472-6807-5-2.pdf Publisher: BioMed Central Ltd..

AB **Restriction enzymes** (REases) are com. reagents commonly used in recombinant DNA technologies. They are attractive models for studying protein-DNA interactions and valuable targets for protein engineering. They are, however, extremely divergent: the amino acid sequence of a typical REase usually shows no detectable similarities to any other proteins, with rare exceptions of other REases that recognize identical or very similar sequences. From structural analyses and bioinformatic studies it has been learned that some REases belong to at least four unrelated and structurally distinct superfamilies of nucleases, PD-DxK, PLD, HNH, and GIY-YIG. Hence, they are extremely hard targets for structure prediction and homol.-based inference of sequence-function relationships and the great majority of REases remain structurally and evolutionarily unclassified. SfiI is a REase which recognizes the interrupted palindromic sequence 5'GGCCNNNN/\NGGCC3' and generates 3 nt long 3' overhangs upon cleavage. SfiI is an archetypal Type **IIF** enzyme, which functions as a tetramer and cleaves two copies of the recognition site in a concerted manner. Its sequence shows no similarity to other proteins and nothing is known about the localization of its active site or residues important for oligomerization. Using the threading approach for protein fold-recognition, we identified a remote relationship between SfiI and BglI, a dimeric Type IIP **restriction enzyme** from the PD-DxK superfamily of nucleases, which recognizes the 5'GCCNNNN/\NGGC3' sequence and whose structure in complex with the substrate DNA is available. We constructed a homol. model of SfiI in complex with its target sequence and used it to predict residues important for dimerization, tetramerization, DNA binding and catalysis. The bioinformatic anal. suggest that SfiI, a Type **IIF** enzyme, is more closely related to BglI, an orthodox Type IIP **restriction enzyme**, than to any other REase, including other Type **IIF** REases with known structures, such as NgoMIV. NgoMIV and BglI belong to two different very remotely related branches of the PD-DxK superfamily: the α-class (EcoRI-like), and the β-class (EcoRV-like), resp. Thus, our anal. provides evidence that the ability to tetramerize and cut the two DNA sequences in a concerted manner was developed independently at least two times in the evolution of the PD-DxK superfamily of REases. The model of SfiI will also serve as a convenient platform for further exptl. analyses.

L17 ANSWER 4 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
2004:816048 Document No. 142:150593 Type IIE and **IIF** **Restriction Endonucleases** Interacting with Two Recognition Sites on DNA. Kirsanova, O. V.; Baskunov, V. B.; Gromova, E. S. (Faculty of Chemistry, Moscow State University, Moscow, 119992, Russia). Molecular Biology (Moscow, Russian Federation, English Edition) (Translation of Molekulyarnaya Biologiya), 38(5), 752-764 (English) 2004. CODEN: MOLBBJ. ISSN: 0026-8933. Publisher: MAIK Nauka/Interperiodica Publishing.

AB A review. Recent studies have shown that **restriction endonucleases** (REs), which are broadly used in genetic engineering and mol. biol., vary not only in nucleotide sequence of the recognition site, but also in the mechanism of their interaction with DNA. This review focuses on type **IIF** and IIE REs, which require simultaneous interaction with two nucleotide sequences for efficient DNA cleavage. Crystal structures of these REs and their complexes with DNA, stepwise

interactions with DNA, catalytic mechanisms of DNA hydrolysis, and DNA looping are considered. Type IIE REs have provided an example of a new type of DNA-protein recognition: two copies of one recognition sequence interact specifically with two different amino acid sequences and two different structural motifs of one polypeptide chain.

L17 ANSWER 5 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
2003:862519 Document No. 140:14293 Diversity of Type II **restriction endonucleases** that require two DNA recognition sites. Mucke, Merlind; Kruger, Detlev H.; Reuter, Monika (Institut fuer Virologie, Medizinische Fakultaet (Charite) der Humboldt-Universitaet zu Berlin, Berlin, D-10098, Germany). Nucleic Acids Research, 31(21), 6079-6084 (English) 2003. CODEN: NARHAD. ISSN: 0305-1048. Publisher: Oxford University Press.

AB Orthodox Type IIP **restriction endonucleases**, which are commonly used in mol. biol. work, recognize a single palindromic DNA recognition sequence and cleave within or near this sequence. Several new studies have reported on structural and biochem. peculiarities of **restriction endonucleases** that differ from the orthodox in that they require two copies of a particular DNA recognition sequence to cleave the DNA. These two sites requiring **restriction endonucleases** belong to different subtypes of Type II **restriction endonucleases**, namely Types IIE, **IIF** and IIS. The authors compare enzymes of these three types with regard to their DNA recognition and cleavage properties. The simultaneous recognition of two identical DNA sites by these **restriction endonucleases** ensures that single unmethylated recognition sites do not lead to chromosomal DNA cleavage, and might reflect evolutionary connections to other DNA processing proteins that specifically function with two sites.

L17 ANSWER 6 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
2002:351486 Document No. 137:75202 Evolutionary relationship between different subgroups of **restriction endonucleases.** Pingoud, Vera; Kubareva, Elena; Stengel, Gudrun; Friedhoff, Peter; Bujnicki, Janusz M.; Urbanke, Claus; Sudina, Anna; Pingoud, Alfred (Institut fur Biochemie, Justus-Liebig-Universitat, Giessen, D-35392, Germany). Journal of Biological Chemistry, 277(16), 14306-14314 (English) 2002. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB The type II **restriction endonuclease** SsoII shows sequence similarity with 10 other **restriction endonucleases**, among them the type IIE **restriction endonuclease** EcoRII, which requires binding to an effector site for efficient DNA cleavage, and the type **IIF restriction endonuclease** NgoMIV, which is active as a homotetramer and cleaves DNA with two recognition sites in a concerted reaction. The authors show here that SsoII is an orthodox type II enzyme, which is active as a homodimer and does not require activation by binding to an effector site. Nevertheless, it shares with EcoRII and NgoMIV a very similar DNA-binding site and catalytic center as shown here by a mutational anal., indicative of an evolutionary relationship between these three enzymes. The authors suggest that a similar relationship exists between other orthodox type II, type IIE, and type **IIF restriction endonucleases.** This may explain why similarities may be more pronounced between members of different subtypes of **restriction enzymes** than among the members of a given subtype.

L17 ANSWER 7 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
2002:314469 Document No. 136:336226 Method for cloning and expression of Bacillus pumilus BpmI **restriction endonuclease** in E. coli. Xu, Shuang-yong; Xiao, Jian-ping; Zhu, Zhenyu (New England Biolabs, Inc., USA). Eur. Pat. Appl. EP 1199365 A2 20020424, 33 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR. (English). CODEN: EPXXDW.

APPLICATION: EP 2001-203826 20011010. PRIORITY: US 2000-2000/693146 20001020.

AB The present invention relates to recombinant DNA which encodes the BpmI **restriction endonuclease** as well as BpmI methyltransferase, and expression of BpmI **restriction endonuclease** from E. coli cells containing the recombinant DNA. BpmI endonuclease is a fusion of two distinct elements with a possible structural domains of restriction-methylation specificity (R-M-S). This domain organization is analogous to the type I restriction-modification system with three distinct subunits, restriction, methylation, and specificity (R, M, and S). Because BpmI is quite distinct to other type IIS **restriction enzymes**, it is proposed that BpmI belongs to a subgroup of type II **restriction enzymes** called type **IIf** (f stands for fusion of restriction-modification- specificity domains). The Type **IIf** group of **restriction enzyme** includes Eco57I, BpmI, GsuI, BseRI and some other **restriction enzymes** that cut downstream sequences at long distance, 10-20 bp downstream of recognition sequence, such as MmeI (N20/N18).

L17 ANSWER 8 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN

1996:256610 Document No. 124:310800 Human serum albumin analogs for preparation of fusion proteins containing a physiologically active peptide to enhance its activities. Higashida, Hideki; Murakami, Kimiko; Hama, Juko; Tsukamoto, Yoko; Isoai, Atsushi; Kumagai, Hiromichi (Asahi Glass Co Ltd, Japan). Jpn. Kokai Tokkyo Koho JP 08051982 A2 19960227 Heisei, 19 pp. (Japanese). CODEN: JKXXAF. APPLICATION: JP 1994-209369 19940811.

AB Human serum albumin to be used as a carrier for physiol. active peptide such as tumor metastasis-inhibiting peptide **IIF**-2 is modified by introducing **restriction endonuclease** recognition sites at its N-terminus, C-terminus, the regions between the 1st and the 2nd domains, or the 2nd and 3rd domains. The coding sequences for the analogs can be prepared by genetic engineering techniques. A chimeric gene encoding the albumin analog and the physiol. active peptide is then prepared for the production of the fusion protein in a desirable host such as Schizosaccharomyces pombe. Preparation of 5 modified genes encoding the albumin analogs was demonstrated. Plasmid pTL2BMI containing a chimeric gene comprised of a gene encoding one of albumin analogs and a **IIF** -2-encoding gene optimized for the expression in S. pombe was prepared and used for the transformation of S. pombe. The biol. activities of the fusion protein purified from the transgenic S. pombe were also demonstrated.

L17 ANSWER 9 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN

1994:319164 Document No. 120:319164 Restriction fragment length polymorphisms in fusarium oxysporum f.sp. dianthi and other fusaria from dianthus species. Manicom, B. Q.; Baayen, R. P. (Inst. Trop. Subtrop. Crops, Nelspruit, 1200, S. Afr.). Plant Pathology, 42(6), 851-7 (English) 1993. CODEN: PLPAAD. ISSN: 0032-0862.

AB DNA restriction fragment length polymorphisms (RFLPs) among 46 isolates of Fusarium oxysporum from Dianthus spp., representing the known range of pathogenicity in carnation, were determined using total DNA digested with the **restriction enzyme** HindIII and a previously described probe, D4. Distinct multiple band RFLP patterns were found, which delineated RFLP groups as follows: (i) F. oxysporum f.sp. dianthi races 1 and 8; (ii) F. oxysporum f.sp. dianthi races 2, 5 and 6; (iii) F. oxysporum f.sp. dianthi race 4; (i.v.) a recently described race of F. oxysporum f.sp. dianthi (wilt-causing isolates from D. caryophyllus formerly classified as F. redolens); (v) wilt-causing isolates from D. barbatus formerly classified as F. redolens and (vi), (vii) and (viii), three further recently described races of F. oxysporum f.sp. dianthi. Isolate groups derived from anal. of RFLPs were consistent with existing and recently described vegetative compatibility groups (VCGs) in F. oxysporum f.sp. dianthi, but not in all cases with races. Isolates of F. oxysporum and F. proliferatum not associated with wilt disease had simpler RFLP patterns (with one exception) that were not associated with VCGs.

L17 ANSWER 10 OF 10 CAPLUS COPYRIGHT 2006 ACS on STN
1979:589616 Document No. 91:189616 Specificity in formation of type II F' plasmids. Hadley, R. G.; Deonier, Richard C. (Mol. Biol., Univ. South. California, Los Angeles, CA, 90007, USA). Journal of Bacteriology, 139(3), 961-76 (English) 1979. CODEN: JOBAAY. ISSN: 0021-9193.

AB Eight new F' plasmids derived from Escherichia coli Hfr strains in which F is integrated at the chromosomal element α3β3 were isolated and subjected to **restriction enzyme**, hybridization, and electron microscope heteroduplex anal. Plasmids carrying extensive amts. of bacterial DNA were produced even though they were obtained by selection for transfer of lac, which is closely linked to F in the parental Hfr strains. Seven plasmids were type II Flac+ proC+ purE+ plasmids, and one was a type I Flac+ proC+ plasmid. Five of the Flac+ proC+ purE+ plasmids contained .apprx.284 kilobases of bacterial DNA which was identical for all 5 within the resolution of the **restriction enzyme** anal. These results indicate that type **II F'** plasmids are the predominant tra+ F' type from this region of the E. coli K-12 chromosome and that the recombination events leading to formation of these plasmids exhibit site specificity.

=> S L7(W)L6
L18 107 L7(W)L6

=> S L18 AND L9
L19 1 L18 AND L9

=> S L19 NOT (L11,L14,L17)
L20 0 L19 NOT ((L11 OR L14 OR L17))

=> S CHANG?;S ALTER?
L21 2100096 CHANG?

L22 886120 ALTER?

=> S (L21,L22) AND L9
L23 153 ((L21 OR L22)) AND L9

=> S L23 AND L5
L24 16 L23 AND L5

=> S L24 NOT (L11,L14,L17)
L25 15 L24 NOT ((L11 OR L14 OR L17))

=> D 1-15 CBIB ABS

L25 ANSWER 1 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN
2006:160775 Engineering a rare-cutting **restriction enzyme**: genetic screening and selection of NotI variants. Samuelson, James C.; Morgan, Richard D.; Benner, Jack S.; Claus, Toby E.; Packard, Stephanie L.; Xu, Shuang-yong (New England Biolabs, Inc., Ipswich, MA, 01938, USA). Nucleic Acids Research, 34(3), 796-805 (English) 2006. CODEN: NARHAD. ISSN: 0305-1048. Publisher: Oxford University Press.

AB **Restriction endonucleases** (REases) with 8-base specificity are rare specimens in nature. NotI from Nocardia otitidis-caviarum (recognition sequence 5'-GCGGCCGC-3') has been cloned, thus allowing for mutagenesis and screening for enzymes with **altered** 8-base recognition and cleavage activity. Variants possessing **altered** specificity have been isolated by the application of two genetic methods. In step 1, variant E156K was isolated by its ability to induce DNA-damage in an indicator strain expressing M.EagI (to protect 5'-NCGGCCGN-3' sites). In step 2, the E156K allele was mutagenized with the objective of increasing enzyme activity towards the **alternative** substrate site: 5'-GCTGCCGC-3'. In this procedure, clones of interest were selected by their ability to eliminate a conditionally toxic substrate vector and induce the SOS response. Thus, specific DNA cleavage

was linked to cell survival. The secondary substitutions M91V, F157C and V348M were each found to have a pos. effect on specific activity when paired with E156K. For example, variant M91V/E156K cleaves 5'-GCTGCCGC-3' with a specific activity of 8.2 + 104 U/mg, a 32-fold increase over variant E156K. A comprehensive anal. indicates that the **cleavage specificity** of M91V/E156K is relaxed to a small set of 8 bp substrates while retaining activity towards the NotI sequence.

L25 ANSWER 2 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN
2005:133483 Document No. 142:312281 The use of prokaryotic DNA methyltransferases as experimental and analytical tools in modern biology. Buryanov, Yaroslav; Shevchuk, Taras (Shemyakin-Ovchinnikov Institute of Bioorganik Chemistry, Pushchino Branch, Russian Academy of Sciences, Moscow, 142290, Russia). Analytical Biochemistry, 338(1), 1-11 (English) 2005. CODEN: ANBCA2. ISSN: 0003-2697. Publisher: Elsevier.

AB A review. Prokaryotic DNA methyltransferases (MTases) are used as exptl. and research tools in mol. biol. and mol. genetics due to their ability to recognize and transfer Me groups to target bases in specific DNA sequences. As a practical tool, prokaryotic DNA MTases can be used in recombinant DNA technol. for in vitro **alteration** and enhancing of **cleavage specificity** of **restriction endonucleases.** The ability of prokaryotic DNA MTases to methylate cytosine residues in specific sequences, which are also methylated in eukaryotic DNA, makes it possible to use them as anal. reagent for determination of the site-specific level of methylation in eukaryotic DNA. In vivo DNA methylation by prokaryotic DNA MTases is used in different techniques for probing chromatin structure and protein-DNA interactions. Addnl. prospects are opened by development of the methods of DNA methylation targeted to predetd. DNA sequences by fusion of DNA MTases to DNA binding proteins. This review will discuss the application of prokaryotic DNA MTases of Type II in the methods and approaches mentioned above.

L25 ANSWER 3 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN
1998:173125 Document No. 128:305523 **Changes** in solvation during DNA binding and cleavage are critical to **altered** specificity of the EcoRI endonuclease. Robinson, Clifford R.; Sligar, Stephen G. (Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, IL, 61801, USA). Proceedings of the National Academy of Sciences of the United States of America, 95(5), 2186-2191 (English) 1998. CODEN: PNASA6. ISSN: 0027-8424. Publisher: National Academy of Sciences.

AB **Restriction endonucleases** such as EcoRI bind and cleave DNA with great specificity and represent a paradigm for protein-DNA interactions and mol. recognition. Using osmotic pressure to induce water release, we demonstrate the participation of bound waters in the sequence discrimination of substrate DNA by EcoRI. **Changes** in solvation can play a critical role in directing sequence-specific DNA binding by EcoRI and are also crucial in assisting site discrimination during catalysis. By measuring the volume **change** for complex formation, we show that at the cognate sequence (GAATTC) EcoRI binding releases about 70 fewer water mols. than binding at an **alternate** DNA sequence (TAATTC), which differs by a single base pair. EcoRI complexation with nonspecific DNA releases substantially less water than either of these specific complexes. In cognate substrates (GAATTC) kcat decreases as osmotic pressure is increased, indicating the binding of about 30 water mols. accompanies the cleavage reaction. For the **alternate** substrate (TAATTC), release of about 40 water mols. accompanies the reaction, indicated by a dramatic acceleration of the rate when osmotic pressure is raised. These large differences in solvation effects demonstrate that water mols. can be key players in the mol. recognition process during both association and catalytic phases of the EcoRI reaction, acting to **change** the specificity of the enzyme. For both the protein-DNA complex and the transition state, there may be substantial conformational differences between cognate and **alternate** sites, accompanied by significant **alterations** in hydration and solvent accessibility.

L25 ANSWER 4 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN
1995:510501 Document No. 122:309654 Heterogeneity in molecular recognition by **restriction endonucleases**: osmotic and hydrostatic pressure effects on BamHI, Pvu II, and EcoRV specificity. Robinson, Clifford R.; Sligar, Stephen G. (Sch. of Chemical Sciences, Univ. of Illinois, Urbana, IL, 61801, USA). Proceedings of the National Academy of Sciences of the United States of America, 92(8), 3444-8 (English) 1995. CODEN: PNASA6. ISSN: 0027-8424. Publisher: National Academy of Sciences.

AB The **cleavage specificity** of the Pvu II and BamHI **restriction endonucleases** is dramatically reduced at elevated osmotic pressure. Relaxation in specificity of these otherwise highly accurate and specific enzymes, previously termed "star activity," is uniquely correlated with osmotic pressure between 0 and 100 atmospheric No other colligative solvent property exhibits a uniform correlation with star activity for all of the compds. tested. Application of hydrostatic pressure counteracts the effects of osmotic pressure and restores the natural selectivity of the enzymes for their canonical recognition sequences. These results indicate that water solvation plays an important role in the site-specific recognition of DNA by many **restriction enzymes.** Osmotic pressure did not induce an analogous effect on the specificity of the EcoRV endonuclease, implying that selective hydration effects do not participate in DNA recognition in this system. Hydrostatic pressure was found to have little effect on the star activity induced by **changes** in ionic strength, pH, or divalent cation, suggesting that distinct mechanisms may exist for these observed **alterations** in specificity. Recent evidence has indicated that BamHI and EcoRI share similar structural motifs, while Pvu II and EcoRV belong to a different structural family. Evidently, the use of hydration water to assist in site-specific recognition is a motif neither limited to nor defined by structural families.

L25 ANSWER 5 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN
1994:211336 Document No. 120:211336 Hydrostatic Pressure Reverses Osmotic Pressure Effects on the Specificity of EcoRI-DNA Interactions. Robinson, Clifford R.; Sligar, Stephen G. (Department of Biochemistry and Chemistry, University of Illinois at Urbana-Champaign, Urbana, IL, 61801, USA). Biochemistry, 33(13), 3787-93 (English) 1994. CODEN: BICHAW. ISSN: 0006-2960.

AB To characterize the role of water in protein-DNA interactions, the authors have studied the specificity of the EcoRI **restriction endonuclease** as a function of osmotic and hydrostatic pressure. The extent of cleavage by the enzyme at noncanonical ("star") sites is shown to depend uniquely upon the osmotic pressure in the reaction as controlled by the addition of a wide variety of neutral solutes. **Alteration** of **cleavage specificity** ("EcoRI* activity") is not uniformly correlated with any other colligative solvent property such as dielec. constant, viscosity, or water concentration The application of hydrostatic pressure reverses the effects of osmotic pressure, restoring the natural selectivity of the enzyme for its canonical site GAATTC. This combination of observations provides compelling evidence that the site-specific recognition of canonical site DNA by EcoRI is mediated by discretely bound water mols. and that the release of these waters induces a fundamental **change** in the specificity of the interaction, leading to cleavage at **alternative** sites. This comprehensive anal. of solvent effects facilitates the unambiguous identification of structurally and functionally specific waters involved in macromol. recognition events.

L25 ANSWER 6 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN
1993:620214 Document No. 119:220214 Cleavage of synthetic substrates containing non-nucleotide inserts by **restriction endonucleases. Change** in the **cleavage specificity** of endonuclease SsoII. Kubareva, E. A.; Petrauskene, O. V.; Karyagina, A. S.; Tashlitskii, V. N.; Nikolskaya, I. I.; Gromova, E. S. (A.N. Belozerskii Inst. Phys. Chem. Biol., Moscow, 119899, Russia).

Nucleic Acids Research, 20(17), 4533-8 (English) 1992. CODEN: NARHAD. ISSN: 0305-1048.

AB A study was made of the interaction between **restriction endonucleases** recognizing CCNGG (N = nucleotide) (SsoII and ScrFI) or CCA/TGG (MvaI and EcoRII) DNA sequences and a set of synthetic substrates containing 1,3-propanediol, 1,2-dideoxy-D-ribofuranose, or 9-[1'-hydroxy-2'-(hydroxymethyl)ethoxy] methylguanine (glG) residues replacing either one of the central nucleosides or dG residues in the recognition site. The non-nucleotide inserts (except for glG) introduced into the recognition site both increase the efficiency of SsoII and **change** its specificity. A cleavage at the noncanonical position takes place, in some cases in addition to the correct ones. Noncanonical hydrolysis by SsoII occurs at the phosphodiester bond adjacent to the point of modification towards the 5'-end. With the guanine base returned (the substrate with glG), the correct cleavage position is restored. ScrFI specifically cleaves all the modified substrates. DNA duplexes with non-nucleotide inserts (except for the glG-containing duplex) are resistant to hydrolysis by MvaI and EcoRII. The peculiarities of recognition by **restriction endonucleases** of 5-membered DNA sequences which have completely or partially degenerated central base pairs are discussed. It is suggested that SsoII forms a complex with DNA in an open form.

L25 ANSWER 7 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN

1990:587065 Document No. 113:187065 Interaction of the EcoRV **restriction endonuclease** with the deoxyadenosine and thymidine bases in its recognition hexamer d(GATATC). Newman, Patrick C.; Williams, David M.; Cosstick, Richard; Seela, Frank; Connolly, Bernard A. (Dep. Biochem., Univ. Southampton, Southampton, SO9 3TU, UK). Biochemistry, 29(42), 9902-10 (English) 1990. CODEN: BICHAW. ISSN: 0006-2960.

AB A set of deoxyadenosine (dA) and thymidine (T) analogs suitable for the study of protein DNA interactions were incorporated into the central d(ATAT) sequence within d(GACGATATCGTC). The individual analogs had one potential protein contact (either a H-bonding group or a CH3 group capable of a van der Waals interaction) deleted. In general, the modified bases did not perturb the overall structure of the dodecamer, enabling results obtained to be simply interpreted in terms of loss of protein DNA contacts. The modified oligodeoxynucleotide set was used to study the recognition of DNA by the EcoRV **restriction endonuclease** [recognition sequence d(GATATC)]. The kcat and Km values for the set were deted., and a comparison with results seen with the parent oligodeoxynucleotide (containing no modified bases) was carried out. Three classes of results were seen. First, some analogs led to no **change** in kinetic parameters, meaning no enzyme contact at the **altered** site. Second, (this was seen for most of the modified oligodeoxynucleotides), a drop in the kcat/Km ratio relative to the parent was observed This came mainly from a decrease in kcat, implying that the endonuclease used the interaction under study to lower the transition-state barrier rather than to bind the substrate. Analyses of these results showed that the drop in kcat/Km was what would be expected for the simple loss of a H-bond or a CH3 contact between the enzyme and the oligodeoxynucleotide. This implies a contact of these types at these sites. Third, some analog-containing oligodeoxynucleotides were not substrates; i.e., the kcat/Km drop was much greater than would be expected for loss of a single H-bond or CH3 contact. These results were interpreted in terms of a cooperative mechanism whereby the loss of one interaction causes a rearrangement at the enzyme active site leading to a consequent loss of further protein substrate contacts. However, in these cases gross structural **changes** in the oligodeoxynucleotide conformation could not be excluded. It was found that the endonuclease made very many interactions to the d(ATAT) sequence within its d(GATATC) recognition site, and these occurred in both the major and minor grooves. The results obtained were used to explain how the enzyme achieves the high degree of **cleavage specificity** for d(GATATC) as compared to all other sequences.

L25 ANSWER 8 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN

1990:511466 Document No. 113:111466 **Altered** specificity of **restriction endonuclease** HinfI. Petronzio, Theresa; Schildkraut, Ira (New England Biolabs, Inc., Beverly, MA, 01915, USA). Nucleic Acids Research, 18(12), 3666 (English) 1990. CODEN: NARHAD. ISSN: 0305-1048.

AB The star activity of HinfI is reported. HinfI isolated in 3 different labs. showed identical **altered** specificity in cleavage of PBR322 DNA; thus, the possibility of this **altered** specificity being the results of another contaminating endonuclease seems unlikely. Star activity may be a general property of **restriction endonucleases** displayed under aberrant reaction conditions.

L25 ANSWER 9 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN

1988:163633 Document No. 108:163633 The use of DNA methylases to **alter** the apparent recognition specificities of **restriction endonucleases.** Nelson, Michael; Schildkraut, Ira (New England Biolabs, Inc., Beverly, MA, 01915, USA). Methods in Enzymology, 155(Recomb. DNA, Pt. F), 41-8 (English) 1987. CODEN: MENZAU. ISSN: 0076-6879.

AB A review, with 9 refs., on the DNA methylase/endonuclease combinations which have generated new **cleavage specificities.** Reagents and procedures are also reviewed.

L25 ANSWER 10 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN

1988:71164 Document No. 108:71164 **Restriction endonuclease** SsoII: reaction with modified substrates. Vinogradova, M. N.; Gromova, E. S.; Uporova, T. M.; Nikol'skaya, I. I.; Shabarova, Z. A.; Debov, S. S. (Mosk. Gos. Univ., Moscow, USSR). Doklady Akademii Nauk SSSR, 295(3), 732-6, 1 plate [Biochem.] (Russian) 1987. CODEN: DANKAS. ISSN: 0002-3264.

AB The recognition and **cleavage specificities** of the **restriction endonuclease** SsoII for various natural and modified, double-stranded oligonucleotides were examined The recognition specificity of this enzyme is known to be 5'-CCAGG-3'. Introduction of structural **changes** in the flanking sequences (an A-A or C-A mismatch) or replacement of the A-T base pair in the recognition sequence (thymidine replaced by 5-fluorodeoxyuridine) induced the selective deceleration in the hydrolysis of the modified strands. Thus, hydrolysis of DNA by SsoII proceeds through a stage in which the enzyme-substrate complex dissocs. after complete breaking of the single strand. In the recognition sequence, replacement of 1 or 2 cytidines in the GC pairs with 5-methyldeoxycytidine rendered the resulting duplexes completely resistant to hydrolysis. On the other hand, the enzyme displayed increased activity for both strands of a duplex in which the AT pair in the recognition sequence was replaced by AU.

L25 ANSWER 11 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN

1986:221249 Document No. 104:221249 **Altered** specificity of the EcoRV **restriction endonuclease.** Halford, Stephen E.; Lovelady, Barbara M.; McCallum, Sarah (Dep. Biochem., Univ. Bristol, Bristol, BS8 1TD, UK). Biochemical Society Transactions, 14(2), 260-1 (English) 1986. CODEN: BCSTB5. ISSN: 0300-5127.

AB The site specificity of **restriction endonuclease** EcoRV was **altered** by **changing** the reaction mixture for DNA digestion from pH 7.5 to 8.5 and adding 10% DMSO. The **altered restriction endonuclease**, EcoRV*, generated a limit digest in contrast to the partial digests obtained by the relaxed specificity of other **restriction endonucleases.** Each addnl. site cleaved by EcoRV* differed from the canonical EcoRV recognition sequence, 5'-GATATC-3', by 1 nucleotide.

L25 ANSWER 12 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN

1986:164182 Document No. 104:164182 Universal **restriction endonucleases**: designing novel **cleavage**

specificities by combining adapter oligodeoxynucleotide and enzyme moieties. Szybalski, Waclaw (McArdle Lab. Cancer Res., Univ. Wisconsin, Madison, WI, 53706, USA). Gene, 40(2-3), 169-73 (English) 1985. CODEN: GENED6. ISSN: 0378-1119.

AB Class IIS **restriction endonucleases** cleave double-stranded (ds) DNA at precise distances from their recognition sequences. A method is proposed which utilizes this separation between the recognition site and the cut site to allow a class IIS enzyme, e.g., FokI, to cleave practically any predetd. sequence by combining the enzyme with a properly designed oligodeoxynucleotide adapter. Such an adapter is constructed from the constant recognition site domain (a hairpin containing the ds sequence) and a variable, single-stranded (ss) domain complementary to the ss sequence to be cleaved (at 9 and 13 nucleotides on the paired strands from the recognition sequence in the example of FokI). The ss sequence designated to be cleaved could be provided by ss phage DNA (e.g., M13), gapped ds plasmids, or supercoiled ds plasmids that were alkali denatured and rapidly neutralized. Combination of all 3 components, namely the class IIS enzyme, the ss DNA target sequence, and the complementing adapter, would result in target DNA cleavage at the specific predetd. site. The target ss DNA could be converted to the precisely cleaved ds DNA by DNA polymerase, utilizing the adapter oligodeoxynucleotide as primer. This novel procedure represents the 1st example of **changing** enzyme specificity by synthetic design. A practically unlimited assortment of new restriction specificities could be produced. The method should have many specific and general applications when its numerous ramifications are exploited.

L25 ANSWER 13 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN

1984:587404 Document No. 101:187404 **Alteration** of apparent **restriction endonuclease** recognition specificities by DNA methylases. Nelson, Michael; Christ, Chantal; Schildkraut, Ira (New England Biolabs, Inc., Beverly, MA, 01915, USA). Nucleic Acids Research, 12(13), 5165-73 (English) 1984. CODEN: NARHAD. ISSN: 0305-1048.

AB An in vitro method of **altering** the apparent **cleavage specificities** of **restriction endonucleases** was developed by using DNA modification methylases. This method was used to reduce the number of cleavage sites for 34 **restriction endonucleases.** In particular, single-site cleavages were achieved for NheI in Adeno-2 DNA and for AccI and HincII in pBR322 DNA by specifically methylating all but one recognition sequence.

L25 ANSWER 14 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN

1980:509646 Document No. 93:109646 Sequence-specific endonuclease Bam HI. Effect of hydrophobic reagents on sequence recognition and catalysis. George, Jay; Blakesley, Robert W.; Chirikjian, Jack G. (Dep. Biochem., Georgetown Univ. Med. Cent., Washington, DC, 20007, USA). Journal of Biological Chemistry, 255(14), 6521-4 (English) 1980. CODEN: JBCHA3. ISSN: 0021-9258.

AB The specificity of cleavage by **restriction endonuclease** BamHI is **altered** in the presence of hydrophobic reagents, such as glycerol and Me2SO. The enzyme with **altered** specificity, designated BamHI.1, generated digestion patterns of various DNAs which were distinct from those generated by BamHI. Cleavage sites recognized in .vphi.X174 RF DNA in the presence of these hydrophobic reagents are not related to the BamHI palindrome. BamHI.1 appears to be an endogenous form of BamHI that can be expressed by **altering** the hydrophobicity of the reaction.

L25 ANSWER 15 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN

1977:513755 Document No. 87:113755 Specificity of cleavage by a restriction nuclease from Bacillus subtilis. Heininger, Kurt; Hoerz, Wolfram; Zachau, Hans G. (Inst. Physiol. Chem., Phys. Biochem. Zellbiol., Univ. Muenchen, Munich, Fed. Rep. Ger.). Gene, 1(5-6), 291-303 (English) 1977. CODEN: GENED6. ISSN: 0378-1119.

AB The **restriction endonuclease** from B. subtilis, which cleaves double-stranded DNA in the middle of the tetranucleotide sequence GGCC, showed a decreased specificity at high endonuclease concns. High pH values, low ionic strength, and high glycerol concns. strongly enhanced the decrease in specificity and also resulted in cleavage of single-stranded DNA. By sequence anal., it was shown that the reduction in specificity corresponds to cleavage predominantly at GC sequences. No comparable **change** in specificity was observed for the **restriction endonuclease** from Haemophilus aegyptius.

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=> E XU S/IN
=> S E3,E23
           286 "XU S"/AU
            97 "XU S Y"/AU
L26        383 ("XU S"/AU OR "XU S Y"/AU)

=> E XU SHUANG Y/AU
=> S E4,E5
            80 "XU SHUANG YONG"/AU
             1 "XU SHUANG YYNG"/AU
L27         81 ("XU SHUANG YONG"/AU OR "XU SHUANG YYNG"/AU)

=> E KOBBE D/AU
=> S E4
L28          1 "KOBBE DANIELA"/AU

=> E ZHU Z/AU
=> S E3
L29        242 "ZHU Z"/AU

=> E ZHU ZHENY/AU
=> S E8
L30         96 "ZHU ZHENYU"/AU

=> E SAMUELSON J/AU
=> S E3-E8
             7 "SAMUELSON J"/AU
             1 "SAMUELSON J C"/AU
             1 "SAMUELSON JACK"/AU
             6 "SAMUELSON JAMES"/AU
            13 "SAMUELSON JAMES C"/AU
             1 "SAMUELSON JAMES CHRISTOPHER"/AU
L31         29 ("SAMUELSON J"/AU OR "SAMUELSON J C"/AU OR "SAMUELSON JACK"/AU
               OR "SAMUELSON JAMES"/AU OR "SAMUELSON JAMES C"/AU OR "SAMUELSON
              JAMES CHRISTOPHER"/AU)


=> S L26,L27,L28,L29,L30,L31
L32        801 (L26 OR L27 OR L28 OR L29 OR L30 OR L31)

=> S L32 AND L5
L33         71 L32 AND L5

=> S L33 AND L8
L34         15 L33 AND L8

=> S L34 NOT (L11,L14,L17,L25)
L35         12 L34 NOT ((L11 OR L14 OR L17 OR L25))

=> D 1-12 CBIB ABS

L35  ANSWER 1 OF 12  CAPLUS  COPYRIGHT 2006 ACS on STN
2005:641699   Document No. 143:148960  Method for engineering strand-specific
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nicking endonucleases from **restriction endonucleases.** **Xu, Shuang-yong; Samuelson, James** (New England Biolabs, Inc., USA). U.S. Pat. Appl. Publ. US 2005158834 A1 20050721, 36 pp. (English). CODEN: USXXCO. APPLICATION: US 2004-13260 20041215. PRIORITY: US 2003-2003/PV530754 20031218.

AB Methods are provided for engineering novel strand-specific nicking endonucleases by an in vivo enrichment of a plasmid library containing a randomly mutagenized **restriction endonuclease** gene. The plasmids contain adjacent to the gene a cleavable or nickable sequence for cleaving or nicking by the endonuclease product of the gene and a second recognition site for a second endonuclease. The plasmid library is used to transform unmodified host cells. Plasmids from the cultured transformed cells may be analyzed by an in vitro assay for nicking and the nicked plasmids pooled and used to transform host cells. The product is then pooled and the single-stranded **specificity** of the endonuclease is then determined The product is either cloned after amplification or identified by use of a selectable marker. Thus, the type IIS **restriction endonuclease** SapI was modified at six positions to form a SapI variant (Nb.SapI) that predominantly nicks the bottom strand between the 4th and 5th nucleotide downstream of the SapI recognition sequence, or at a single position (E250K) to form a variant (Nt.SapI) that exclusively nicks the top strand between the 1st and 2nd nucleotide downstream of the SapI recognition sequence.

L35 ANSWER 2 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN

2005:547042 Document No. 143:73870 Preparation of strand-specific nicking endonucleases by site directed mutagenesis. **Zhu, Zhenyu; Xu, Shuang-yong** (New England Biolabs, Inc., USA). U.S. Pat. Appl. Publ. US 2005136462 A1 20050623, 45 pp. (English). CODEN: USXXCO. APPLICATION: US 2004-13235 20041215. PRIORITY: US 2003-2003/PV531064 20031219.

AB Methods are provided for identifying novel strand-specific nicking endonucleases by means of in vitro backcrosses of mutagenized **restriction endonuclease** genes with their wild-type counterpart and identifying the resulting nicking endonucleases by their cleavage activity and their strand **specificity.** The preparation process includes the transformation in E. coli without methylase protection, the ligation of N-terminal region of wild type and the mutated C-terminal half, second transformation with the synthesized DNA with methylase protection. Nicking endonucleases identified by this method include Nt.BsaI, Nb.BsaI, Nt.BsmAI, Nb.BsmAI and Nt.NBsmBI have been presented as examples.

L35 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN

2005:406958 Document No. 143:111396 Implications for Switching **Restriction Enzyme Specificities** from the Structure of BstYI Bound to a BglII DNA Sequence. Townson, Sharon A.; **Samuelson, James C.; Xu, Shuang-yong;** Aggarwal, Aneel K. (Structural Biology Program, Department of Physiology & Biophysics, Mount Sinai School of Medicine, New York, NY, 10029, USA). Structure (Cambridge, MA, United States), 13(5), 791-801 (English) 2005. CODEN: STRUE6. ISSN: 0969-2126. Publisher: Cell Press.

AB Summary: The type II **restriction endonuclease** BstYI recognizes the degenerate sequence 5'-RGATCY-3' (where R = A/G and Y = C/T), which overlaps with both BamHI (GGATCC) and BglII (AGATCT), and thus raises the question of whether BstYI DNA recognition will be more BamHI-like or BglII-like. The authors present here the structure of BstYI bound to a cognate DNA sequence (AGATCT). The authors find the complex to be more BglII-like with similarities mapping to DNA conformation, domain organization, and residues involved in catalysis. However, BstYI is unique in containing an extended arm subdomain, and the mechanism of DNA capture has both BglII-like and BamHI-like elements. Further, DNA recognition is more minimal than BglII and BamHI, where only two residues mediate recognition of the entire core sequence. Taken together, the structure reveals a mechanism of degenerate DNA recognition and offers insights into the possibilities and limitations in altering **specificities** of closely related **restriction enzymes.**

L35 ANSWER 4 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
2005:15871 Document No. 142:88792 Recycled mutagenesis of **restriction endonucleases**, BsoBI and PvuII, toward enhanced catalytic activity. **Xu, Shuang-Yong; Zhu, Zhenyu**; Riggs, Paul D.; Hsieh, Pei-Chung (USA). U.S. Pat. Appl. Publ. US 2005003420 A1 20050106, 23 pp. (English). CODEN: USXXCO. APPLICATION: US 2004-874527 20040623. PRIORITY: US 2003-PV484256 20030701.

AB A method is described for increasing the activity of **restriction endonuclease** mutants that have altered binding or cleavage activities. **Restriction endonuclease** variants can carry one or more amino acid substitutions that change substrate **specificity** and at the same time decrease the enzyme catalytic activity. A method is described for isolating derivs. of the endonuclease variants by subjecting them to addnl. rounds of mutagenesis and screening in a dinD::lacZ indicator strain, such that second-site mutations within the nucleotide coding sequence of the endonuclease are obtained that increased the enzyme specific activity.

L35 ANSWER 5 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
2004:317293 Document No. 140:334621 Crystal structure of BstYI at 1.85 Å resolution: A thermophilic **restriction endonuclease** with overlapping **specificities** to BamHI and BglII. Townson, Sharon A.; **Samuelson, James C.**; Vanamee, Eva Scheuring; Edwards, Thomas A.; Escalante, Carlos R.; **Xu, Shuang-yong**; Aggarwal, Aneel K. (Department of Physiology and Biophysics, Structural Biology Program, Mount Sinai School of Medicine, New York, NY, 10029, USA). Journal of Molecular Biology, 338(4), 725-733 (English) 2004. CODEN: JMOBAK. ISSN: 0022-2836. Publisher: Elsevier.

AB The crystal structure of **restriction endonuclease** BstYI, an "intermediate" type II **restriction endonuclease** with overlapping sequence **specificities** to BamHI and BglII, is reported. BstYI, a thermophilic endonuclease, recognizes and cleaves the degenerate hexanucleotide sequence 5'-RGATCY-3' (where R = A or G and Y = C or T), cleaving DNA after the 5'-R on each strand to produce 4 base (5') staggered ends. The crystal structure of free BstYI was solved at 1.85 Å resolution by multiwavelength anomalous dispersion (MAD) phasing. Comparison with BamHI and BglII revealed a strong structural consensus between all 3 enzymes mapping to the α/β core domain and residues involved in catalysis. Unexpectedly, BstYI also contained an addnl. "arm" substructure outside of the core protein, which enabled the enzyme to adopt a more compact, intertwined dimer structure compared with BamHI and BglII. This arm substructure may underlie the thermostability of BstYI. Putative DNA recognition residues were identified and the authors speculate as to how this enzyme achieves a "relaxed" DNA **specificity**.

L35 ANSWER 6 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
2004:190664 Document No. 140:370801 Engineering Strand-specific DNA Nicking Enzymes from the Type IIS **Restriction Endonucleases** BsaI, BsmBI, and BsmAI. **Zhu, Zhenyu; Samuelson, James C.**; Zhou, Jing; Dore, Andrew; **Xu, Shuang-yong** (New England Biolabs, Inc., Beverly, MA, 01915, USA). Journal of Molecular Biology, 337(3), 573-583 (English) 2004. CODEN: JMOBAK. ISSN: 0022-2836. Publisher: Elsevier.

AB More than 80 type IIA/IIS **restriction endonucleases** with different recognition **specificities** are now known. In contrast, only a limited number of strand-specific nicking endonucleases are currently available. To overcome this limitation, a novel genetic screening method was devised to convert type IIS **restriction endonucleases** into strand-specific nicking endonucleases. The genetic screen consisted of four steps: (1) random mutagenesis to create a plasmid library, each bearing an inactivated endonuclease gene; (2) restriction digestion of plasmids containing the wild-type and the mutagenized endonuclease

gene; (3) back-crosses with the wild-type gene by ligation to the wild-type N-terminal or C-terminal fragment; (4) transformation of the ligated DNA into a pre-modified host and screening for nicking endonuclease activity in total cell culture or cell exts. of the transformants. Nt.BsaI and Nb.BsaI nicking endonucleases were isolated from BsaI using this genetic screen. In addition, site-directed mutagenesis was carried out to isolate BsaI nicking variants with minimal double-stranded DNA cleavage activity. The equivalent amino acid substitutions were introduced into BsmBI and BsmAI **restriction endonucleases** with similar recognition sequence and significant amino acid sequence identity and their nicking variants were successfully isolated. This work provides strong evidence that some type IIS **restriction endonucleases** carry two sep. active sites. When one of the active sites is inactivated, the type IIS **restriction endonuclease** may nick only one strand.

L35 ANSWER 7 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
2003:571173 Document No. 139:113669 Alteration of **restriction endonuclease specificity** by genetic selection.
Samuelson, James C.; Xu, Shuang-Yong (New England Biolabs, Inc., USA). PCT Int. Appl. WO 2003060152 A2 20030724, 51 pp.
DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US542 20030109.
PRIORITY: US 2002-2002/PV347403 20020110.

AB Methods and compns. are provided for altering the DNA recognition and cleavage characteristics of an endonuclease without prior knowledge of the endonuclease's three-dimensional structure and/or amino acid residues responsible for activity and/or **specificity.** A protocol developed to achieve in vivo selection process includes one or more of the following steps: (1) generating a mutated endonuclease library within an expression vector or plasmid; (2) introducing the endonuclease library into prokaryotic host cells pre-modified with a non-cognate pattern of methylation; (3) pooling survivors and plasmid DNA from the cells; (4) isolating active endonuclease clones by culturing individual colonies for a short time at a low temperature and preparing plasmid DNA from these cultures; (5) introducing individual plasmid isolates (or pooled plasmid DNA) into a DNA damage indicator strain which is pre-modified with the same pattern of methylation as in step (2). The stringent selection method allows rapid screening of an estimated 107 variants in one round. The method is exemplified by increasing the substrate **specificity** of Bacillus stearothermophilus Y406 endonuclease BstYI (5'-RGATCY-3') to single site recognition (5'-AGATCT-3').

L35 ANSWER 8 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
2003:482984 Document No. 139:257111 Isolation of BsoBI **Restriction Endonuclease** Variants with Altered Substrate **Specificity**
. **Zhu, Zhenyu**; Zhou, Jing; Friedman, Alan M.; **Xu, Shuang-yong** (New England Biolabs, Inc., Beverly, MA, 01915, USA).
Journal of Molecular Biology, 330(2), 359-372 (English) 2003. CODEN: JMOBAK. ISSN: 0022-2836. Publisher: Elsevier Science Ltd..

AB BsoBI is a thermophilic **restriction endonuclease** that cleaves the degenerate DNA sequence C/PyCGPuG (where / = the cleavage site and Py = C or T, Pu = A or G). In the BsoBI-DNA co-crystal structure the D246 residue makes a water-mediated hydrogen bond to N6 of the degenerate base adenine and was proposed to make a complementary bond to O6 of the alternative guanine residue. To investigate the substrate **specificity** conferred by D246 and to potentially alter BsoBI **specificity**, the D246 residue was changed to the other 19 amino acids. Variants D246A, D246C, D246E, D246R, D246S, D246T, and D246Y were purified and their cleavage activity determined Variants D246A, D246S, and D246T display 0.2% to

0.7% of the wild-type cleavage activity. However, the substrate **specificity** of the three variants is altered significantly. D246A, D246S, and D246T cleave CTCGAG sites poorly. In filter binding assays using oligonucleotides, wild-type BsoBI shows almost equal affinity for CTCGAG and CCCGGG sites. In contrast, the D246A variant shows 70-fold greater binding affinity for the CCCGGG substrate. Recycled mutagenesis was carried out on the D246A variant, and revertants with enhanced activity were isolated by their dark blue phenotype on a dinD::lacZ DNA damage indicator strain. Most of the amino acid substitutions present within the revertants were located outside the DNA-protein interface. This study demonstrates that endonuclease mutants with altered **specificity** and nonlethal activity can be evolved towards more active variants using a laboratory evolution strategy.

L35 ANSWER 9 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
2002:482232 Document No. 137:212814 Directed evolution of **restriction endonuclease** BstYI to achieve increased substrate **specificity**. **Samuelson, James C.**; **Xu, Shuang-yong** (New England Biolabs, Beverly, MA, 01915, USA). Journal of Molecular Biology, 319(3), 673-683 (English) 2002. CODEN: JMOBAK. ISSN: 0022-2836. Publisher: Elsevier Science Ltd..

AB **Restriction endonucleases** have proven to be especially resistant to engineering altered substrate **specificity**, in part, due to the requirement of a cognate DNA methyltransferase for cellular DNA protection. Thermophilic **restriction endonuclease** BstYI recognizes and cleaves all hexanucleotide sequences described by 5'-R↓GATCY-3' (where R = A or G and Y = C or T). The recognition of a degenerate sequence is a relatively common feature of the >3000 characterized **restriction endonucleases**. However, very little is known concerning substrate recognition by these enzymes. The authors' objective was to investigate the substrate **specificity** of BstYI by attempting to increase the **specificity** to recognition of only AGATCT. By a novel genetic selection/screening process, 2 BstYI variants were isolated with a preference for AGATCT cleavage. A fundamental element of the selection process is modification of the Escherichia coli host genomic DNA by the BglII N4-cytosine methyltransferase to protect AGATCT sites. The amino acid substitutions resulting in a partial change of **specificity** were identified and combined into one superior variant designated NN1. BstYI variant NN1 displayed a 12-fold preference for cleavage of AGATCT over AGATCC or GGATCT. Moreover, cleavage of the GGATCC sequence was no longer detected. This study provides further evidence that laboratory evolution strategies offer a powerful alternative to structure-guided protein design.

L35 ANSWER 10 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
2001:176305 Document No. 134:292043 **Restriction enzyme** BsoBI-DNA complex: a tunnel for recognition of degenerate DNA sequences and potential histidine catalysis. Van der Woerd, Mark J.; Pelletier, John J.; **Xu, Shuang-Yong**; Friedman, Alan M. (Department of Biological Sciences, Purdue University, West Lafayette, IN, 47907, USA). Structure (Cambridge, MA, United States), 9(2), 133-144 (English) 2001. CODEN: STRUE6. ISSN: 0969-2126. Publisher: Cell Press.

AB Background: **Restriction endonucleases** form a diverse family of proteins with substantial variation in sequence, structure, and interaction with recognition site DNA. BsoBI is a thermophilic **restriction endonuclease** that exhibits both base-specific and degenerate recognition within the sequence CPyCGPuG. Results: The structure of BsoBI complexed to cognate DNA has been determined to 1.7 Å resolution, revealing several unprecedented features. Each BsoBI monomer is formed by inserting a helical domain into an expanded EcoRI-type catalytic domain. DNA is completely encircled by a BsoBI dimer. Recognition sequence DNA lies within a 20 Å long tunnel of protein that excludes bulk solvent. Interactions with the specific bases are made in both grooves through direct and water-mediated hydrogen bonding. Interaction with the degenerate position is mediated by a purine-specific hydrogen bond to N7, ensuring **specificity**, and water-mediated H bonding to the purine N6/O6 and pyrimidine N4/O4, allowing

degeneracy. In addition to the conserved active site residues of the DXn(E/D)ZK **restriction enzyme** motif, His253 is positioned to act as a general base. Conclusions: A catalytic mechanism employing His253 and two metal ions is proposed. If confirmed, this would be the first example of histidine-mediated catalysis in a **restriction endonuclease.** The structure also provides two novel examples of the role of water in protein-DNA interaction. Degenerate recognition may be mediated by employing water as a hydrogen bond donor or acceptor. The structure of DNA in the tunnel may also be influenced by the absence of bulk solvent.

L35 ANSWER 11 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
1997:748056 Document No. 128:137042 The Tsp45I restriction-modification system is plasmid-borne within its thermophilic host. Wayne, Jay; Holden, Megan; **Xu, Shuang-yong** (New England Biolabs Inc., 32 Tozer Road, Beverly, MA 01915, USA). Gene, 202(1/2), 83-88 (English) 1997. CODEN: GENED6. ISSN: 0378-1119. Publisher: Elsevier Science B.V..

AB Thermus species YS45 harbors two small cryptic plasmids of 5.8 (pTsp45s) and approx. 12 kb (pTsp45l). Plasmid pTsp45s has been entirely sequenced, revealing three significant ORFs. In addition to a previously reported thermophilic plasmid-encoded replication protein (Rep), pTsp45s contains two genes for the Tsp45I methyltransferase (M.Tsp45I) and **restriction endonuclease** (Tsp45I). These two converging genes (tsp45IM and tsp45IR) overlap by 4 bp at their stop codons within an XbaI site. M.Tsp45I (413 aa, 47.0 kDa, recognizing 5'-GTSAC-3') is highly homologous to other m6A-methyltransferases, especially M.EcaI (recognizing 5'-GGTNACC-3'). Tsp45I (332 aa, 37.4 kDa, cleaving 5'-↓GTSAC-3') is not homologous to M.Tsp45I, or to other **restriction endonucleases.** Recombinant Tsp45I is stably produced in E. coli, and cleaves DNA at 65°C with the same **specificity** as the native enzyme. Therefore, the thermophilic Tsp45I restriction- modification system is plasmid-borne within its native host.

L35 ANSWER 12 OF 12 CAPLUS COPYRIGHT 2006 ACS on STN
1995:680055 Document No. 123:106510 Isolation of temperature-sensitive mutants of the BamHI **restriction endonuclease.** Fomenkov, Alexey; **Xu, Shuang-yong** (New England Biolabs Inc., Beverly, MA, 01915, USA). Gene, 157(1/2), 303-10 (English) 1995. CODEN: GENED6. ISSN: 0378-1119. Publisher: Elsevier.

AB Two heat-sensitive R·BamHI mutants, T157I and P173L, and one cold-sensitive R·BamHI mutant, T114I, were isolated after chemical mutagenesis of the bamhIR gene that codes for the **restriction endonuclease** BamHI (R·BamHI). The thermosensitivity of T114I, T157I and P173L is revealed by the 102-103 lower plating efficiency at the non-permissive temperature of strains bearing these alleles. The conditional-lethal phenotype can be rescued in vivo and display reduced phage restriction activity. The mutant enzymes induce the SOS response in vivo and display reduced phage restriction activity. The P173L protein, when expressed at 30 °C and purified, showed reduced thermostability at 65°C. T157I and P173L mutants yield different intermediates during partial trypsin digestion. The conditional-lethal BamHI mutants could be used to deliver in vivo DNA cleavage and for further isolation of relaxed-**specificity** mutants.

EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
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L2	2727	METHYLASE	US-PGPUB; USPAT	ADJ	ON	2006/04/04 10:24
L3	218809	DOMAIN	US-PGPUB; USPAT	ADJ	ON	2006/04/04 10:24
L4	106885	CLEAVAGE	US-PGPUB; USPAT	ADJ	ON	2006/04/04 10:24
L5	127267	SPECIFICITY	US-PGPUB; USPAT	ADJ	ON	2006/04/04 10:24
L6	1062	L4 ADJ L5	US-PGPUB; USPAT	ADJ	ON	2006/04/04 10:25
L7	268	L4 ADJ L3	US-PGPUB; USPAT	ADJ	ON	2006/04/04 10:25
L8	233	L5 ADJ L3	US-PGPUB; USPAT	ADJ	ON	2006/04/04 10:25
L9	3	L6 AND L7 AND L8	US-PGPUB; USPAT	ADJ	ON	2006/04/04 10:27
L10	2220	IIG OR (II ADJ G)	US-PGPUB; USPAT	ADJ	ON	2006/04/04 10:28
L11	3181	IIF OR (II ADJ F)	US-PGPUB; USPAT	ADJ	ON	2006/04/04 10:29
L12	4	L6 AND L10	US-PGPUB; USPAT	ADJ	ON	2006/04/04 10:29
L13	7	L6 AND L11	US-PGPUB; USPAT	ADJ	ON	2006/04/04 10:29
L14	11	L12 OR L13	US-PGPUB; USPAT	ADJ	ON	2006/04/04 10:31
L15	2660355	CHANGE OR CHANGING OR ALTER OR ALTERS	US-PGPUB; USPAT	ADJ	ON	2006/04/04 10:33
L16	999	L15 AND L6	US-PGPUB; USPAT	ADJ	ON	2006/04/04 10:33
L17	840	L16 AND L1	US-PGPUB; USPAT	ADJ	ON	2006/04/04 10:33
L18	57	L15 SAME L6	US-PGPUB; USPAT	ADJ	ON	2006/04/04 10:34
L19	50	L18 AND L1	US-PGPUB; USPAT	ADJ	ON	2006/04/04 10:34